QTL Analysis of Root Architecture Traits and Low Phosphorus Tolerance in an Andean Bean Population

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ABSTRACT

Tolerance to low P soils is a desirable trait in common bean (*Phaseolus vulgaris* L.) cultivars grown in acid-weathered soils. Genetic variability in response to P-deficient soils exists in the Andean gene pool. G19833, a low P–tolerant indeterminate Andean landrace, has been evaluated for quantitative trait loci (QTL) and tolerance to low P in combination with Mesoamerican parents. Our goal was to expand our understanding of phenotypic traits and QTL from G19833 expressed in an Andean background. An $F_{5:7}$ recombinant inbred line (RIL) population developed from two Andean bean genotypes, G19833 and AND696, a determinate line susceptible to low P soils, was examined under low and high soil P levels in replicated field trials. Phosphorus uptake was reduced by 70% and yield by 60% in low P compared to high P treatments. A linkage map was developed with 11 linkage groups and a total length of 1105 cM. Quantitative trait loci for root growth, seed yield, seed P content, and P use efficiency colocalized near the *fin* gene on linkage group B1. Quantitative trait loci for root growth traits, seed yield, and P uptake colocalized on B11. Quantitative trait loci for root length density (cm cm^{-3}) and root surface area (cm^2) did not colocalize with QTL for P uptake. This study indicates that root traits did not play an important role in tolerance to low P soil in an Andean × Andean cross, contrary to previous results with the same P-efficient Andean parent (G19833) crossed to a Mesoamerican bean genotype.

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Abbreviations: AFLP, amplified fragment length polymorphisms; HP, high phosphorus treatment; LP, low phosphorus treatment; PCR, polymerase chain reaction; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RIL, recombinant inbred line; RLD, root length density; RSA: root surface area; SSR, simple sequence repeat.

Common bean (*Phaseolus vulgaris* L.) is an important grain legume, produced annually on 20 million hectares worldwide. The highest production and consumption of common bean occurs in Latin America (5.6 million metric tons) and Africa (2.8 million metric tons) (FAO, 2006). One of the major constraints to bean production in developing countries is low available soil P (Wortmann et al., 1998). An estimated 43% of the land in the tropics has acid-weathered soil, especially those in the Andisol, Ultisol, and Oxisol orders. These soils adsorb or fix P so that 70 to 90% of P fertilizer applied reacts with iron or aluminum to form compounds of low solubility (Sanchez and Salinas, 1981).

Tolerance to low P soils is defined as the ability to produce plant growth and yield in relation to the amount of available P (Lynch and Beebe, 1995) and can occur by two distinct routes: acquisition efficiency and utilization efficiency. Acquisition efficiency reflects the plants' ability to extract P from the environment. Phosphorus acquisition has been shown to be related to root system traits that increase the root surface area (RSA) and allow capture of more P

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from the soil (Gahoonia and Nielsen, 2003). Utilization efficiency is the superior ability of a plant to convert acquired P into plant biomass and yield and is related to reduced P requirement in plant tissue (Lynch and Beebe, 1995).

Tolerance to P deficiency has been identified in bean lines from diverse genetic backgrounds in both the Andean and Mesoamerican gene pools (Beebe et al., 1997). A number of Peruvian landraces of Andean origin, including G19839 and G19833, have been found to be effective producers in low P soils (Yan et al., 1995a, 1995b). Inheritance of tolerance to low P has been challenging to study, and major genotype × environment interactions for seed yield necessitate the use of indicators of tolerance to low P soils other than seed yield. A number of physiological traits correlated with tolerance to low P soils have been identified (Beebe et al., 1997). Research has shown P efficiency to be related to greater P uptake from the soil in G19833. Root system phenotypes that exhibit shallower basal root angle, greater total root length and RSA, and root length of basal roots in the top 3 cm have been shown to enhance P uptake in P-limiting environments (Bonser et al., 1996; Liao et al., 2004; Beebe et al., 2006).

The measurement of root traits in the field is laborious and root growth is very plastic even with small changes in the soil environment (Snapp et al., 1995), thus complicating the selection of plants with desired root traits. Quantitative trait loci (QTL) analysis is a powerful tool for understanding genetic variation and control of complex traits such as tolerance to low P soils. The identification of QTL serves as a starting point for marker-assisted selection, which offers potential to improve traits with low heritabilities or difficult-to-measure traits without relying on phenotypic selection (Collard et al., 2005).

Studies using G19833 as the P-efficient parent in crosses with DOR364, a Mesoamerican small-seeded red bean as the inefficient parent, have identified QTL for root traits that colocalize with QTL for P uptake efficiency (Beebe et al., 2006; Liao et al., 2004). Quantitative trait loci for both root length and P uptake in low P soil were found in the same region of linkage group B4. Additional QTL for specific root length and P uptake under the same environmental conditions colocalized to a region of B10 (Beebe et al., 2006). Liao et al. (2004) also identified QTL on B4 in a growth pouch assay of percentage of basal roots in the top 3 cm that colocalized with QTL for P uptake in the field in the G19833/DOR364 population.

The identification of QTL for root architecture traits with QTL for P uptake efficiency in crosses between the Andean and Mesoamerican gene pools raises the question if similar mechanisms for P efficiency would be observed in an Andean × Andean cross using the same P efficient parent, G19833. Our objectives were to (i) construct a linkage map for a recombinant inbred line (RIL) population derived from G19833 and AND696, two Andean common bean genotypes contrasting for tolerance to low P soils, (ii) use the map to identify QTL affecting phenotypic traits related to tolerance to low P soils in an Andean genetic background, and (3) examine effectiveness of previously identified QTL from G19833 related to tolerance to low P soils in a cross with a Mesoamerican parent.

MATERIALS AND METHODS

Plant Material

The common bean genotypes G19833 and AND696 from the Andean gene pool were used as parents to develop 75 $F_{5:7}$ RILs. F_2 plants from the initial cross were advanced to the F_5 generation by single seed descent. G19833 is a Peruvian landrace with an indeterminate growth habit; large yellow and red mottled seed with an average 100 seed weight of 46 g and tolerance to low P soils (Yan et al., 1995a, 1995b; Beebe et al., 2006). AND696 is a CIAT improved line from the race Nueva Granada with a determinate growth habit and large red and cream mottled seed with an average 100 seed weight of 51 g. AND696 has been identified as susceptible to low P soils (CIAT, 2000). The RILs developed from G19833 and AND696 segregated for determinacy, with 52 determinate and 23 indeterminate lines. Growth habit in common bean is controlled by a single gene, *fin* (Norton, 1915). The number of determinate and indeterminate lines deviated significantly from a 1:1 ratio expected for a single gene trait. This deviation is likely due to the selection of RILs with uniform days to maturity, which favored determinate lines during SSD advance.

Field Trials

In 2000 and 2005, the 75 RILs, AND696 (2005 only), G19833 (2000 only), and two check varieties, G4017 (Carioca) and G16140, were planted in Darien, Colombia (3°54′ N, 76°30′ W, 1485 m above sea level, average yearly temperature 20°C, average relative humidity 80%, average yearly rainfall 1288 mm in 189 d of rain per year) with three replications at two soil P levels, low P and high P. G4017 is an indeterminate (type III) Brazilian land race of the Mesoamerican gene pool that exhibits tolerance to low P soils (Miller et al., 2003). G16140 is a Peruvian landrace with an indeterminate growth habit (Type III), identified as susceptible to low soil fertility (Singh et al., 2003).The soil at the field site is an Udand with native soil P of 2 mg kg^{-1} determined by Bray II extraction (Olsen and Sommers, 1982), pH 5.6, and 10.6% organic matter (dicromate–sulfuric acid oxidation). Several years of fertility management to generate high and low P plots in this location resulted in plots having markedly different native P availability with average P concentration of 64.3 g kg^{-1} and 1.7 g kg^{-1} for high and low P plots, respectively. Before planting, the high P plot received 45 kg ha^{-1} P and the low P plot received 7.5 kg ha^{-1} P as triple super phosphate. Seed was hand-planted at 0.05 m spacing in four-row plots. Each row was 3 m in length. The middle two rows were planted to the genotypes of interest and the outer two rows were border rows of easily distinguished cream-seeded cultivar BAT 477. The border rows were included to improve uniformity in soil P availability to the different genotypes, and to aid in identification of genetic material. To prevent zinc and boron deficiency typical of these soils, 0.6 kg ha^{-1} of $ZnSO_4$ and 0.6 kg ha^{-1} of borax [$Na_2B_4O_2(H_2O)_{10}$]

were applied as a foliar feed every week after emergence for three consecutive weeks. Phosphorus-free fungicides and pesticides were applied as needed (Ochoa et al., 2006).

Plant Measurements (2005 Only)

A number of plant and root traits were measured in 2005 relevant to tolerance to low P soil. Adventitious root number was counted on two plants for each P treatment and in each replication at 21 d after planting. This was done by excavating entire root systems from the ground. At mid-pod fill shoots from 50-cm row length (which was equivalent to 5–10 plants) were harvested and oven dried at 70°C until free of moisture. Dry weights were recorded and shoots were ground to a fine powder in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) with a 60-mesh screen. Plant tissue samples were Kjeldahl acid-digested and subsequently measured for concentration of P according to the method of Murphy and Riley (1962) using colorimetric spectrometry. Phosphorus uptake per plant was determined by multiplying P shoot concentration by plant dry weight of a 50-cm row divided by number of plants in the row (mg plant^{-1}). It is important to note that P uptake in this study was measured in the shoot only and P in the roots was not measured, so the values calculated slightly underestimate P uptake by the whole plant.

At mid-pod fill, a single root sample adjacent to plant stems was taken per plot from each of three replications using a 7.1-cm-diameter soil auger, to a depth of 16 cm. Roots were separated from soil and washed with water. Soil was dried and weighed to determine volume. Cleaned roots were placed in a transparent plastic tray (22 by 28 by 6.5 cm) and covered with water. The tray containing roots was then scanned with a flat bed scanner with a top and bottom light source at 300 dpi to produce a two-dimensional scanned image. The images were analyzed with WinRHIZO software (Regent Instruments Inc., Quebec, Canada) and root length (cm), RSA (cm^2), and average root diameter (mm) of the samples was determined. Following image capture, roots were dried at 65°C for 5 d and dry weights were determined. Root length density (RLD) was calculated by dividing root length by soil volume sampled (cm cm^{-3}) and specific root length was calculated by dividing root length by root dry weight (cm mg^{-1}).

Harvest and Seed Measurements (2005 and 2000)

At maturity, seeds were hand-harvested. Seeds were dried and seed yield was determined at 12% moisture. Seed weight (of 100 seed) was measured at 12% moisture for the 2000 and 2005 plantings. A subsample of seed was analyzed for total P concentration. Five grams of seed of each treatment for each of the three replications were cleaned with distilled water and dried. Samples were then freeze dried to remove all moisture. Freeze-dried seed samples were ground to a fine powder with a Retsch mill (E Kurt Retsch GMBH, Haan, Germany). Seed samples were Kjeldahl acid digested and subsequently measured for concentration of P according to the method of Murphy and Riley (1962) using colorimetric spectrometry. Seed P content was calculated by multiplying seed P concentration by 100 seed weight (mg 100 seed^{-1}).

Statistical Analysis

Statistical analysis of phenotypic traits measured was conducted with SAS for Windows V8 (SAS Institute, Cary, NC). The command PROC GLM was used to determine P treatment, genotype, and interaction effects via a two way analysis of variance. When a single trait was measured in more than 1 yr, analysis was not conducted across years and each year's data was considered as a unique trait. The command PROC CORR was used to determine Pearson correlation coefficients among variables.

DNA Isolation and Molecular Marker Analysis

Leaf tissue was harvested from five plants of greenhouse grown seed of G19833, AND696, and the 75 $F_{5:7}$ RILs. Total DNA was extracted from leaf tissue with 24:1 chloroform/isoamyl alcohol according to the method of Edwards et al. (1991). DNA was quantified with a fluorometer (Hoefer DyNA Quant 200, San Francisco, CA). Molecular markers screened in the study for polymorphisms between the parents G19833 and AND696 included 213 simple sequence repeats (SSRs) (Metais et al., 2002; Blair et al., 2003; Gaitan-Solis et al., 2002; Yu et al., 2000; Caixeta et al., 2005; Grisi et al., 2007), 50 random amplified polymorphic DNA (RAPD) (Integrated DNA Technologies, Inc., Coralville, IA) and 41 amplified fragment length polymorphisms (AFLP) primer combinations.

Polymerase chain reaction (PCR) amplification of SSR markers was performed with 2 μL of DNA diluted to 20 ng μL^{-1}, 0.2 μL of primer, 0.15 μL of *Taq* polymerase, 2.5 mM $MgCl_2$, 1.2 μL PCR buffer, and 0.12 μL of a 10 mM mix of dNTPs. Polymerase chain reaction was conducted in a 96-well PTC-100 Programmable Thermal Controller (MJ Research, Inc., Waltham, MA) that was programmed for one cycle of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 47°C, and 1 min at 72°C, and a final extension step at 72°C for 5 min. Double-stranded DNA product was denatured for 5 min at 94°C before loading onto 4% polyacrylamide gels and DNA bands were visualized with silver nitrate staining according to the procedure of Blair et al. (2003).

Random amplified polymorphic DNA amplification was performed with 5 μL of DNA diluted to 10 ng μL^{-1}, 1 μL of primer (Integrated DNA Technologies, Inc., Coralville, IA), 0.3 μL of *Taq* polymerase, 2.5 mM $MgCl_2$, 25 μL commercial PCR buffer, and 0.5 μL of a 10 mM mix of dNTPs. Polymerase chain reaction was conducted in a 96-well PTC-100 Programmable Thermal Controller (MJ Research, Inc.) that was programmed for two cycles of 1 min at 91°C, 15 s at 42°C, 70 s at 72°C; 38 cycles of 15 s at 91°C, 15 s at 42°C, 70 s at 72°C; 5 min at 72°C. A total of 20 μL of PCR product was loaded onto 1.5% agarose (in 0.5× TBE buffer) gels with ethidium bromide and run in electrophoresis chambers with 1.5 L 0.5× TBE buffer for 3 h at 110 V. Polymerase chain reaction amplification products were visualized under UV light. Random amplified polymorphic DNA markers were named according to the primer used and the molecular weight of the band in kilobases.

Amplified fragment length polymorphisms reactions were performed according to a commercially available AFLP Analysis System I kit (Invitrogen Corp., Carlsbad, CA) with the modification that the preamplification PCR product (primer + 1 base) was run in 1% agarose gel and diluted 1:50. The amplification (primer +3 bases) was performed with 5 μL of the diluted DNA and 5 μL of

Mix I (primers and dNTPs) and 10 μL Mix II (10× buffer, $MgCl_2$, and DNA polymerase). The PCR conditions were one cycle at 94°C for 30 s, 65°C for 60 s, 72°C for 60 s; 13 cycles with annealing temperatures lowered 0.7°C per cycle; 23 cycles at 94°C for 30 s, 56°C for 60 s, 72°C for 60 s. Polymerase chain reaction amplification products were separated on 4% polyacrylamide gels and DNA bands were visualized with silver nitrate according to the procedure of Blair et al. (2003). The AFLPs were named by combining the last two selective bases ligated to *Eco*RI with the last two selective bases ligated to *Mse*I in the amplification step. The individual bands were numbered such that the largest molecular weight band was numbered 01 and each band was consecutively numbered by decreasing size. Those AFLP markers near QTL are also described with band size in basepairs (Fig. 1).

Genetic Map Construction and QTL Analysis

Linkage analysis was conducted with the software JoinMap 3.0 for Windows (Van Ooijen and Voorrips, 2002) set to Kosambi's map function. Kosambi's mapping function assumes the existence of interference that is negatively related to recombination frequency. Analysis was conducted with marker data of 167 molecular markers segregating in

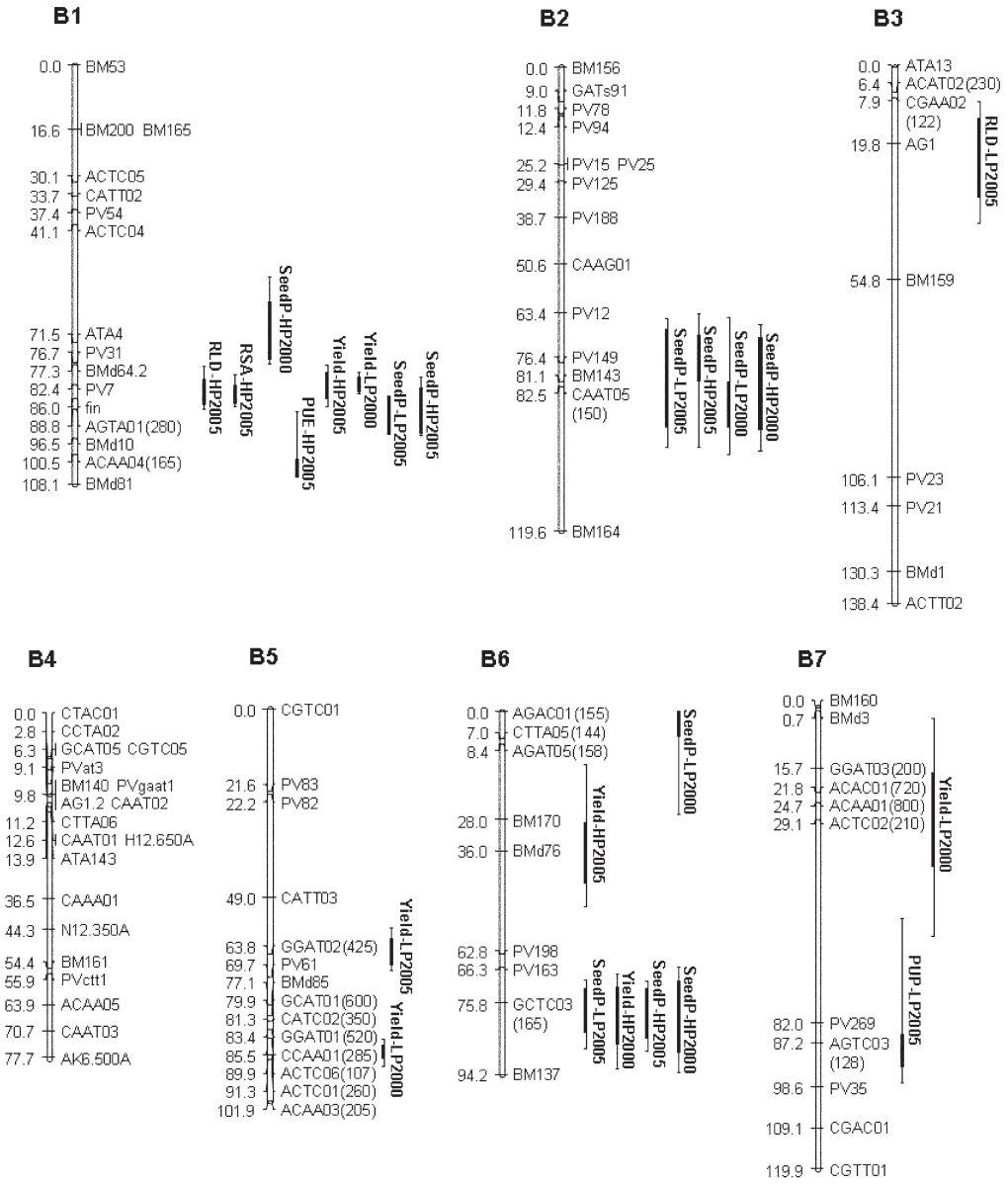

Figure 1. Common bean linkage map and quantitative trait loci (QTL) locations developed from 75 recombinant inbred lines (RILs) of the $F_{5:7}$ population AND696/G19833. The map contains random amplified polymorphic DNA (RAPD; named as a letter–number combination followed by fragment size in base pairs, with the letter–number combinations: AA19, AK6, H12, I6, L4, M12, 012, and R4), amplified fragment length polymorphisms (AFLP; named as four letters and two numbers with first two letters of AA, AC,AG, CA, CC, CG, CT, GC, GG), simple sequence repeat (SSR; first two to three letters: AG, ATA, BM, GAT, ME, PV) markers (Metais et al., 2002; Blair et al., 2003; Gaitan-Solis et al., 2002; Yu et al., 2000; Caixeta et al., 2005; Grisi et al., 2007) and one phenotypic marker (*fin*) for a total of 1105 cM on 11 linkage groups. Those AFLP markers near a QTL also show the fragment size in base pairs next to the marker name. Abbreviations: high P treatment (HP); low P treatment (LP); root length density (RLD); root surface area (RSA); P use efficiency (PUE); P uptake (PUP). The horizontal line across QTL indicates the position of highest LOD peak as identified with composite interval mapping. The outer interval (thin line) is the 2-LOD threshold and the inner interval (thick line) is the 1-LOD threshold.

the population of RILs. Mapping parameters were set to recombination frequency smaller than 0.300 and a log of odds (LOD) score larger than 3.0. Parameters were relaxed to a recombination frequency of smaller than 0.45 and a LOD score greater than 2.0 to join unlinked markers and to join fragmented linkage groups containing SSRs previously identified to specific linkage groups (Blair et al., 2003). LOD scores are equal to log (L_1/L_0), where L_1 is the likelihood for the alternative hypothesis and L_0 is the likelihood of the null hypothesis. A LOD score of 3 means the alternative hypothesis is 1000 times more likely than the null hypothesis. Linkage groups were identified according to location of SSR markers (Blair et al., 2003) and were named according to the core bean linkage map (Freyre et al., 1998).

Quantitative trait loci analysis was conducted with the genetic map developed with the JoinMap program and with the phenotypic means for each RIL collected from the field study. The computer software program QTL Cartographer version 2.5 for Windows (Wang et al., 2006) was used to identify QTL for RLD, RSA, P uptake, P use efficiency, seed yield, and seed P content. The composite interval mapping feature set to a window size of 10 cM, walkspeed of 1 cM, and with forward and backward regression model was used to identify QTL. Composite interval mapping involves the use of maximum likelihood estimates and linear regression to identify QTL within marker intervals. Significant QTL were considered by defining the LOD score at $P = 0.01$ after 1000 permutation tests (Churchill and Doerge, 1994).

RESULTS

Linkage Map

DNA polymorphism levels with the molecular markers used in this study were low to moderate between the Andean parents G19833 and AND696. Of the 213 SSR markers screened, all of which were developed for use in common bean, 31% were polymorphic between the parent lines. The linkage map, developed from the 167 marker loci with 64 SSRs, 11 RAPD, 91 AFLP, and one phenotypic marker screened in the G19833/AND696 RIL population, spanned 1105 cM. Common bean has 11 linkage groups corresponding to the genome's 11 chromosomes. In this study, all 11 linkage groups were identified according to the bean consensus map, based on the placement of SSR markers (Freyre et al., 1998; Blair et al., 2003) (Fig. 1).

QTL Identification

Using composite interval mapping, 29 QTL were identified for six traits (RLD, RSA, P uptake, P use efficiency, seed yield, and seed P content) on eight linkage groups (Fig. 1). Twenty-eight percent of the QTL were clustered on linkage group B1 and affected more than one trait. An additional 17% of the QTL were located on B11 and also affected multiple traits. Individual QTL explained 8 to 33% of the phenotypic variation, and total phenotypic variation explained for any one trait ranged from 11 to 56% (Table 1).

Seed Yield

Significant differences in seed yield of the RIL population were observed between the high P treatment (HP) and low P treatment (LP) (Table 1). In 2005, the mean yield was 597 kg ha^{-1} in LP and 1345 kg ha^{-1} in HP. Yield differences between P treatments in 2000 were similar to those observed in 2005 (Table 2). The differences in yield between P treatments demonstrate the effects of limiting P on plant growth in this population. In both years, there

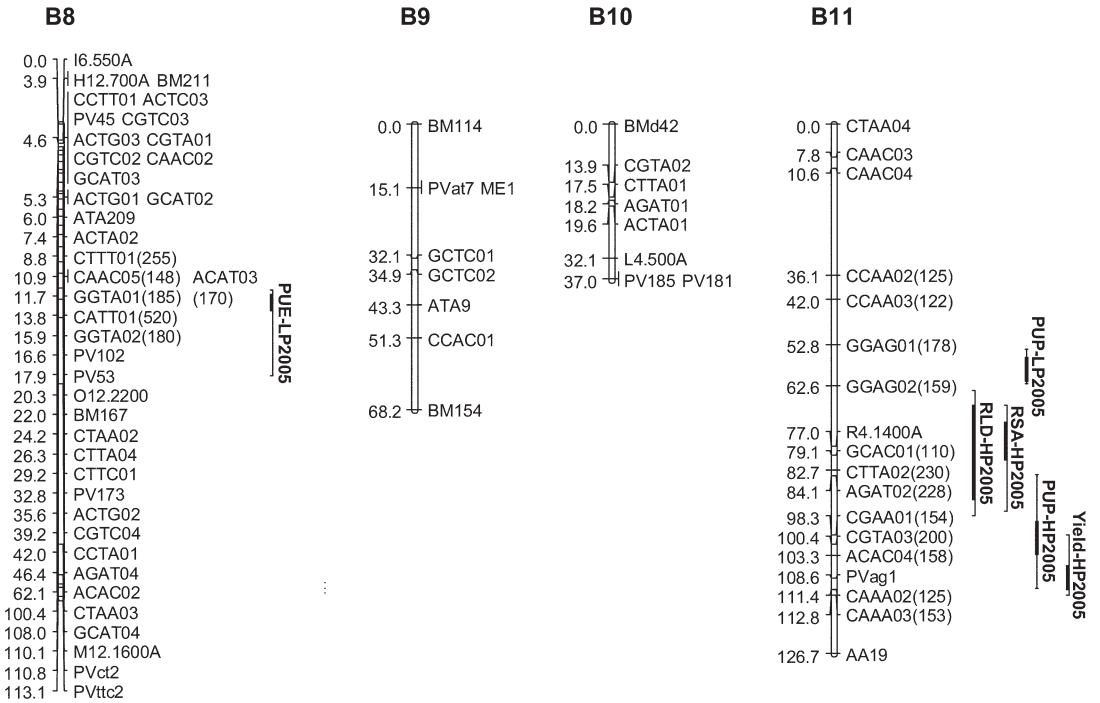

Figure 1. Continued.

was significant genotypic variability for yield and in 2005 there was a significant genotype × P treatment interaction (Table 1).

A total of eight QTL were identified for seed yield. Quantitative trait loci identified for yield in HP in 2005 and LP in 2000 both map to the same region of B1 and the increased effects on yield for these QTL were derived from different parents (Table 3). The remaining six QTL for seed yield were distinct from each other. In 2000, all QTL for yield in both HP and LP were conferred by G19833, whereas in 2005, G19833 was the source of two QTL, and AND696 was the source of two other QTL.

Phosphorus Uptake

Significant differences in P uptake were observed between P treatments such that mean P uptake among RILs was more than three times higher in HP than LP (Tables 1 and 2). Genotypic variability was observed for P uptake among RILs and significant genotype × P treatment interactions were observed for this trait (Table 1).

A QTL, conferred by G19833, was detected on B7 for P uptake in LP. Quantitative trait loci for P uptake in HP and LP were identified on B11 and were conferred by AND696 (Table 3).

Root Architecture

A selection of root traits were evaluated in the 2005 growing season to determine their variability in the population and importance for P uptake. Mean squares from analysis of variance identified significant genotypic differences in RLD, specific root length, RSA, and average root diameter (Table 1). Further inspection of these traits indicated only RLD and RSA were significantly different among the RILs in LP; RLD, RSA, and average root diameter were significantly different among the RILs in HP (Table 2).

The mean RLD of the RILs was greater in the LP (1.36 cm cm^{-3}) than in HP (1.08 cm cm^{-3}). Quantitative trait loci identified for RLD in HP were distinct from the QTL identified for the same trait in LP (Table 3). The QTL found for RSA in HP mapped to the same regions of B1 and B11 as QTL for RLD in HP. The increased effect of each of the root traits was derived from G19833 (Table 1).

Phosphorus Use Efficiency

Phosphorus use efficiency was defined in this study as the amount of seed yield per unit of P taken up by the plant (g seed mg P^{-1}). Phosphorus use efficiency was greater across RILs in HP than in LP. Variability for P use efficiency between P treatments and among genotypes was observed (Table 1). A QTL was detected on B8 for P use efficiency in LP derived from G19833 that explained 18% of the phenotypic variation (Table 3).

Seed Phosphorus Content

Phosphorus treatment influenced seed P content such that when grown in LP, seeds contained less P than when grown in HP in both 2000 and 2005 (Table 2). Phosphorus content was correlated with seed yield ($r = 0.30$; $P < 0.0001$) in 2005 in LP, but it was not correlated with yield in any other P treatment × year combination. Quantitative trait loci identified for this trait were identified on B1, B2, and B6 across P treatments and years. In all but one case an increase in seed P content was derived from G19833 (Table 3).

DISCUSSION

In developing the linkage map a low to moderate level of polymorphism was observed. Moderate polymorphism levels have been seen in intra–gene pool crosses such as this one between two Andean genotypes. A recent study of SSR polymorphism levels compared 33 markers in 123 common bean genotypes from the Andean gene pool, and the polymorphism levels ranged from 45 to 57% (Blair et al., 2007). Twenty-seven of the thirty-three markers screened by Blair et al. (2007) were also screened between G19833 and AND696 and 44% were polymorphic.

Seed yield was reduced by over 55% in LP as compared to HP. Most of the QTL for yield identified did not colocalize across P treatment or year. Both years had similar average yield ratios in LP/HP of about 40%.

Table 1. Analysis of variance of root traits, traits related to P uptake and use, and seed yield in a population of recombinant inbred lines derived from the AND696/G19833 cross grown in two soil P treatments: high and low soil P in Darien, Colombia, in 2005 and 2000. All values are for the 2005 season unless otherwise indicated.

Source	df	Mean squares									
		Root length density	Specific root length	Root surface area	Avg. root diam.	P uptake	P use efficiency	Seed P content		Seed yield	
								2005	2000	2005	2000
Genotype (G)	74	0.56***	21*	2210***	0.008***	263***	0.04**	2092***	1672***	161,674***	465,740***
Soil P (P level)	1	9.06***	31 ns†	691 ns	0.065***	120,770***	5.26***	324,541***	205,516***	62,929,473***	88,298,547***
G × P level	74	0.31 ns	13 ns	1538 ns	0.006 ns	220***	0.02 ns	420 ns	673***	103,183**	211,912 ns

*Significance at $P < 0.05$.

**Significance at $P < 0.01$.

***Significance at $P < 0.001$.

†ns, not significant.

Table 2. Mean plant growth traits in high and low P soil conditions for parents AND696 and G19833 and the means and ranges of recombinant inbred lines (RILs) developed from the parents. Means are also included for the check varieties, G4017 and G16140. The experiment was planted in 2000 and 2005 in Darien, Colombia. All values are for the 2005 season unless otherwise indicated. Mean values are of three replications. *P* value indicates level of significant genotypic differences among the RILs for each trait.

Traits	P level	Parents		RILs			Check varieties	
		AND696	G19833	Mean	Range	*P* value	G4017	G16140
Root length density, cm cm^{-3}	High	1.35	–	1.08	0.57–2.30	0.0008	0.70	2.1
	Low	1.59	–	1.36	0.59–2.59	0.0281	0.66	1.54
Specific root length, cm mg^{-1}	High	11.1	–	8.4	3.3–17.6	0.5144	5.7	10.5
	Low	9.71	–	8.8	4.0–18.7	0.3887	4.4	8.9
Root surface area, cm^2	High	87.9	–	71.7	33–147.4	0.0040	53.4	120
	Low	60.3	–	74.0	32.2–156.5	0.0289	37.7	80.6
Avg. root diam., mm	High	0.24	–	0.33	0.25–0.53	0.0128	0.37	0.28
	Low	0.26	–	0.31	0.24–0.40	0.3464	0.32	0.29
P uptake, mg plant^{-1}	High	36.5	–	46.7	25–92	0.0010	70.9	63.4
	Low	14.5	–	13.7	6.6–26.1	0.0112	8.9	16.5
P use efficiency, g seed mg P^{-1}	High	0.33	–	0.29	0.11–0.50	0.0016	0.29	0.15
	Low	0.27	–	0.51	0.25–0.78	0.2787	0.79	0.41
Seed P (2005), mg 100 seed^{-1}	High	197.4	–	193	146–261	<0.0001	110.7	254.1
	Low	146.6	–	139	97–181	<0.0001	87.8	201.3
Seed P (2000), mg 100 seed^{-1}	High	–	162.2	160	107–256	<0.0001	93.3	296.4
	Low	–	–	116	76–151	<0.0001	68.4	142.5
Seed yield (2005), kg ha^{-1}	High	1347	–	1345	582–1962	<.0001	2096	1046
	Low	465	–	597	323–944	0.0542	830	714
Seed yield (2000), kg ha^{-1}	High	–	1080	1516	681–2871	<0.0001	3038	1670
	Low	–	649	642	268–1064	<0.0001	1344	977

Interestingly, the yield increase explained by the QTL on B1 was conferred by G19833 in LP and by AND696 in HP. The region of B1 where these QTL were located was equivalent to the position of the *fin* gene that is responsible for determinacy. The AND696/G19833 population segregated for determinacy, with the efficient parent, G19833 exhibiting indeterminate plant growth and the inefficient parent, AND696, exhibiting determinate plant growth.

Linkage group B1 has previously been shown to be important in the common bean domestication syndrome (Koinange et al., 1996) and carries both the *fin* gene and photoperiod sensitivity genes (*Ppd*) which are responsible for earliness. Furthermore, seed size traits have been identified on this linkage group, near these domestication genes (Koinange et al., 1996). The importance of the region on B1 near the *fin* gene suggests for our study either a pleiotropic effect of this gene on yield, or linkage of this gene with other genes that are important determinants of yield. The observation that this region of the bean genome had an opposite effect on seed yield under high and low soil P levels may be an indication that growth habit itself is a key mechanism to improve tolerance to low P soils, perhaps through indirect effects on increased maturity that provides more time for P uptake from the soil. It has been observed that the cluster of domestication genes on B1 have pleiotropic effects on other traits in common bean, including plant morphology, leafhopper (*Empoasca fabae* Harris and *E. kraemeri* Ross and Moore) resistance (Murray et al., 2004), and white mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] avoidance (Miklas et al., 2001).

The parental source of tolerance to low P soil used in this study, G19833, has been shown to be efficient in P uptake (Beebe et al., 2006). This finding is supported in this study by the identification of a QTL for P uptake in LP derived from G19833 that mapped nearby a QTL for yield in LP (2000) also derived from G19833. This QTL for P uptake on B7 explained 10% of the variation for this trait. Although this value is low and may not warrant marker-assisted selection for this region of the genome, it underscores the complexity of the low P tolerance trait.

Our study further examined specific root traits in relation to P uptake, to pinpoint mechanisms of P uptake efficiency and to aid in selection criteria for plant improvement. The population afforded an opportunity to study the inheritance of root architecture in field-grown plants in a solely Andean background, which has not been attempted before since most QTL studies have used inter–gene pool populations (e.g., Beebe et al., 2006; Liao et al., 2004; Yan et al., 2004). Despite the plastic nature of root growth it was possible to detect genetic differences in these traits in this population. Quantitative trait loci identified for RLD and RSA were conferred by G19833. These variables

measure root coverage per volume of soil and are indicators of explorative capacity of the plant. Root architecture traits that increase the volume of soil exploration may in turn increase the capture of immobile P ions by a plant root system (Gahoonia and Nielsen, 2003). Explorative capacity in the upper soil profile becomes very important for ion uptake in soils with low levels of P.

Table 3. Putative quantitative trait loci (QTL) for seed traits identified from recombinant inbred lines developed from AND696/G19833 cross grown under high (HP) and low (LP) soil P conditions in Darien, Colombia, in 2000 and 2005. Linkage group location and nearest marker to the peak LOD value are given for each QTL.

Traits	Linkage group	Nearest marker	LOD score†	R^2 CIM‡	Additivity§
Root length density					
LP 2005	B3	AG1	2.97	0.14	–0.18
HP 2005	B1	PV7	4.36	0.19	–0.15
	B11	GCAC01	4.23	0.17	–0.14
Root surface area					
HP 2005	B1	*fin*	6.21	0.26	–14
	B11	R4.140A	3.39	0.13	–9.2
P uptake					
LP 2005	B7	AGTC03	2.5	0.10	–1.4
	B11	GGAG01	5.51	0.33	2.8
HP 2005	B11	CGAA01	2.95	0.13	4.5
P use efficiency					
LP 2005	B8	CCTA01	3.88	0.18	–0.08
HP 2005	B1	BMd10	2.95	0.11	0.02
Seed yield					
LP 2005	B5	GGAT02	4.21	0.19	74.8
HP 2005	B1	PV7	6.4	0.24	144
	B6	PV198	3.15	0.11	–92
	B11	PVag1	3.41	0.12	–111
LP 2000	B1	PV7	7.56	0.32	–148
	B5	ACTC06	3.04	0.12	–84
	B7	ACAC01	3.04	0.12	–72
HP 2000	B6	GCTC03	2.76	0.11	–146
Seed P content					
LP 2005	B1	AGTA01	5.88	0.22	–8.4
	B2	CAAT05	4.88	0.15	–6.6
	B6	GCTC03	5.01	0.16	–6.5
HP 2005	B1	fin	5.14	0.17	–10.9
	B2	PV149	2.71	0.08	–7.3
	B6	GCTC03	5.75	0.21	–11.4
LP 2000	B2	CAAT05	3.73	0.12	–4.9
	B6	AGAC01	3.71	0.12	4.9
HP 2000	B1	ATA4	6.38	0.18	–13.1
	B2	CAAT05	5.49	0.14	–10.3
	B6	GCTC03	3.28	0.09	–8.3

†LOD, Log of odds.

‡Proportion of the phenotypic variance explained by QTL at test site using composite interval mapping (CIM).

§Effects of substituting a single allele from one parent to another. Positive values indicate that allelic contribution is from AND696 and negative from G19833.

A QTL for specific root length in greenhouse-grown RILs from the population G19833/DOR364 was previously identified in the same region of B1 (Beebe et al., 2006) as the QTL described here under high P for RLD and RSA. Additional QTL for root traits in the G19833/DOR364 population identified by Beebe et al. (2006) were found at the same region of B3 as QTL identified in this study under LP for RLD. These QTL have been shown to be associated with taproot length in greenhouse-grown RILs (Beebe et al., 2006), and a QTL for taproot root hair length in plants grown in solution culture (Yan et al., 2004). It is significant that QTL identified for root traits in this study on B1 and B3 were also sites of QTL for root traits in DOR364/G19833, demonstrating the ability to consistently identify QTL for root growth traits in field-grown plants derived from G19833 in both Andean and Mesoamerican genetic backgrounds.

In the DOR364/G19833 population G19833 was the source of QTL for root length and P uptake in low soil P which colocalized on B4 (Beebe et al., 2006). In the AND696/G19833 population G19833 was also the source of a QTL for P uptake, this however was found on a different linkage group (B7) than that identified in the Mesoamerican background and also did not colocalize with QTL for root traits.

Quantitative trait loci for RLD and RSA in HP found on B11 colocalized with a QTL for yield in HP soil in 2005. The source QTL for yield was AND696 whereas QTL for the root traits came from G19833. This suggests that while a dense shallow root system may be useful in low-nutrient environments, in P-sufficient environments the added energy allocated to the root system may contribute to reduced yield potential. The region of B11 for which QTL were identified has been important in other QTL studies as well. On B11 near SSR marker PV-ag001 a QTL for adventitious root length in high P soil was identified in the G2333/G19833 RIL population (Ochoa et al., 2006). Quantitative trait loci for days to flower and pods per plant have also been identified on B11 also near the SSR marker PV-ag001 in an Andean (ICA Cerinza) × wild bean (G24404) population (Blair et al., 2006). This region of B11 appears to have an influence on plant growth and maturity that should be studied to determine the underlying genes that influence many diverse traits.

Whereas identifying QTL for P uptake efficiency is important to agriculture in naturally low P soils, QTL for P utilization efficiency may have a broader application. Improvement in genetics of P use efficiency can potentially reduce fertilizer need on a wide range of soil types. In this study, P use efficiency was defined as the amount of seed yield per unit of P uptake by the plant, and P use efficiency was not significant in the low P soil. A QTL originating from G19833 was identified for this trait on B8. This is in contrast to findings with DOR364/G19833,

where P use efficiency was not identified as a mechanism of tolerance to low soil P (Beebe et al., 2006). However, preliminary QTL studies with the P-efficient bean line G21212 identified P use efficiency as a factor in tolerance to P-deficient soils (Miklas et al., 2006).

Phosphorus content of the seed was studied here in relationship to LP tolerance. In P-limiting environments, greater levels of P in the seed may aid early plant growth (Lynch and Beebe, 1995). Nutrients stored in the seed are important for germination and early plant growth (Liptay and Arevalo, 2000). Increased seed P content partially compensated for the negative effects of LP soils on early seedling growth in wheat (*Triticum aestivum* L.) through increases in shoot dry weight, leaf size, and root length (De Marco, 1990). The positive phenotypic correlation was observed between seed P content and yield in LP in 2005 ($r = 0.30$) was also observed in the co-localization of QTL for seed P and seed yield in HP 2000 on B6 near the AFLP marker GCTC03, both derived from G19833.

In summary, P-uptake QTL were identified in this Andean bean population that may play a minor role in tolerance to LP soils. Differences in root growth traits were observed in the population, and while the P-tolerant parent contributed to increased RLD and RSA in the topsoil, these traits were not associated with increased P uptake. Based on the results of this study, it appears that attempting to breed for larger or denser root systems will not have the benefit in improving tolerance to LP soils in an Andean background as it may in a Mesoamerican background.

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